Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

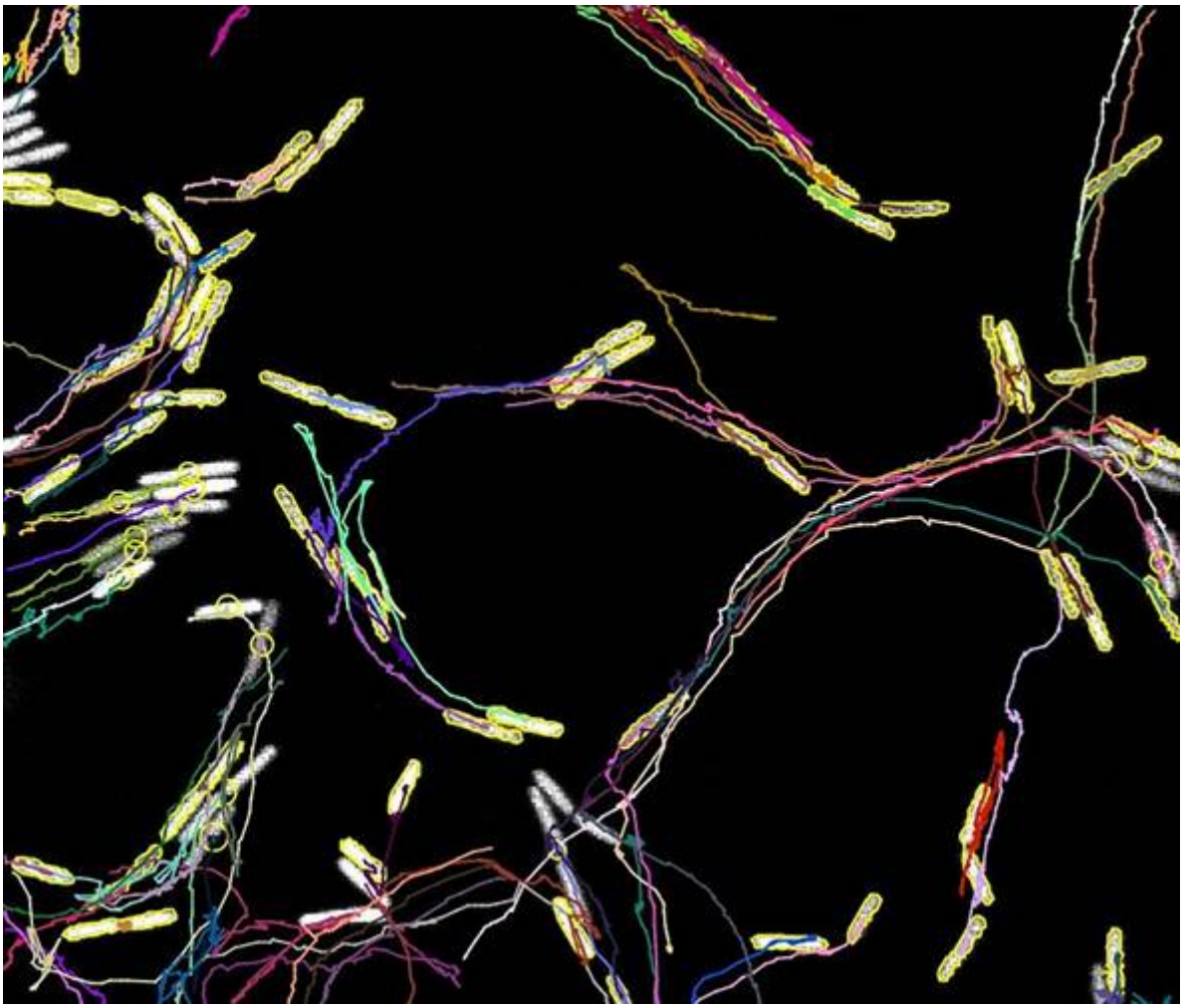

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

Peroxide stress alters *Bacillus subtilis* extracellular vesicles composition

Barnech Carolina[1,3] - Cerny Natacha[1,3] - Malamud Florencia[1,3] - Coluccio Leskow Federico* [1,3] - Cimolai María Cecilia* [1,2]

1) Departamento de Ciencias Básicas, Universidad Nacional de Luján, Luján, Buenos Aires, Argentina.
2) Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), Buenos Aires, Argentina.
3) Comisión Nacional de Investigación Científica y Tecnológica (CONICET), Buenos Aires, Argentina.
Contacto: carobarnech88@gmail.com

Bacillus subtilis is a Gram-positive spore-forming bacterium that can be isolated from diverse environments and has various biotechnological applications. Bacterial extracellular vesicles (EV) are nanoscale structures released to the extracellular medium, composed of lipids, proteins and nucleic acids. Gram-positive EV functions and biological roles are yet not completely understood. We hypothesize that secreted EV could serve as communication particles in which signals (proteins, nucleic acids) are protected from extracellular proteases and nucleases. Thus, our aim was to characterize *B. subtilis* derived EV produced under peroxide stress conditions and to evaluate their possible biological role in cell communication. EV isolation was performed by several steps of centrifugation, filtration and ultracentrifugation of B. subtilis 168 culture supernatants in control (EVC) and stress conditions (EVH; peroxide stress: H_2O_2 58 ?M, non-letal dose). The resulting EV were analyzed by NTA and were found to exhibit similar biophysical properties, specifically in terms of size distribution (median size EVC: 120 ± 2; EVH: 122 ± 1 nm; n=3) and zeta potential (EVC: -42.7 ± 2; EVH: -43.6- ± 1 mV). Despite these similarities, the EVs populations displayed differences in their abundance (EVC: 2.83x10^10; EVH:1.53x10^11 mean of particles/ml n=3), as well as in their protein (EVC: 69 ± 7; EVH:151 ± 31 ?g/ml) and lipid content (EVC: 0.29 ± 0.1; EVH:1.6 ± 0.6 ?g DOPC/10 ?l). Since EVs biological functions are not completely understood, we tested the effect of these EVs on *B. subtilis* 168 growth and observed that the treatment with a tenfold concentrated preparation of EVc (n=3) resulted in curves with lower plateau, indicative of growth inhibition. In contrast, treatment with EVH did not produce any observable impact on bacterial growth. On the other hand we tested the ability of EVs to prevent cellular stress, since we have previously observed a high representation of stress-related proteins in EV protein cargo by Orbitrap-nanoHPLC screening proteomics. For this we analyzed EV capacity to modify cell response to stress: *B. subtilis* cells were treated with EV, washed and loaded with DCFDA (2',7'-dichlorofluorescin diacetate; 50?M), a probe thar reflects intracellular oxidation state. EVs (2.5 fold concentrated) treated cells resulted in

lower dye oxidation in the presence of H_2O_2 0.05 % (control: 0.63 ± 0.03; H_2O_2 : 0.99 ±0.01; EVC: 0.47±0.12; EVH: 0.55±0.07 EVC + H_2O_2 : 0.69 ±0.24; EVH+ H_2O_2 : 1.03± 0.09; AU; n=2-3. H_2O_2 vs EVC + H2O2 ANOVA post test p=0.07), reflecting a possible protective effect only with EVC. In summary we could show that *B. subtilis* EV secreted into the extracellular medium are different in number and cargo when peroxide stress is induced and this may lead to different EV biological functions.

Palabras clave: Bacillus subtilis - extracellular vesicles - gram positive bacteria - stress